1078-178 Application of Synthetic CDK Inhibition (Flavopiridol) as a Novel Therapeutic Strategy to Effectively Limit Common Pathways of In-Stent Restenosis: Characterization of Molecular Effects and Applicability on Drug Coated Stents

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The cellular component of in-stent restenotic lesions is mainly comprised of proliferated and migrated coronary artery smooth muscle cells (CASMC). Early cell cycle inhibition represents an attractive therapeutic strategy. Cyclin-dependent kinases (CDK) trigger and coordinate transitions between different phases of the cell cycle. Flavopiridol (FLA) is a highly effective synthetic CDK inhibitor. The main purpose of the study was to determine the efficacy and molecular effects of FLA in limiting mitogen induced CASMC proliferation and migration as well as to examine the effects of FLA coated stents on the prevention of in-stent restenosis. Results: FLA displayed potent anti-proliferative effects in CASMC, the IC50 was determined at 75 nM (BrdU ELISA, cell counting). Accordingly, CASMC CDK2, 4 and PCNA protein levels were dose dependently downregulated. Cell cycle analysis by propidium iodine flow cytometry revealed a G1 arrest in Flavopiridol treated CASMC. Given the potent anti-restenotic effects of Sirolimus coated stents, upregulated levels of endogenous CDK inhibitors such as p21 and particularly p27 may be critical for effective anti-restenotic therapy. FLA lead to a dose dependent increase of p21 and p27 protein levels in CASMC. FLA prevented p27 degradation. At concentrations of 100nM, there was neither evidence of FLA induced cytotoxicity (LDH release ELISA) nor apoptosis (ssDNA ELISA). A Boyden chamber assay revealed significant reduction of CASMC migration towards a fibronectin gradient by more than 50% at 100 nM. FLA coated stents lead to significant inhibition of CASMC proliferation in an in vitro model (FLA 137+/-12 cells, control 454+/-48, p<0.01). Initial results in an ongoing study using a rat carotid stenting model indicate reduced neointima formation in animals treated with FLA coated stents. Conclusions: Flavopiridol displays potent anti-restenotic effects. CDK inhibition by novel synthetic compounds such as Flavopiridol via drug coated stents may be an effective approach to limit neointima formation following stent placement and highlights the importance of early cell cycle inhibition as an effective tool to limit restenotic processes.

1078-179 Interferon Gamma Receptor 1 88 G/A Polymorphism and Restenosis Following Coronary Artery Stenting

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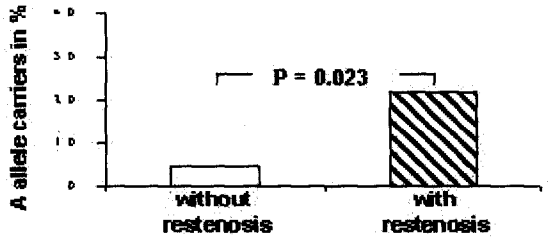
Background: Recently, upregulation of interferon gamma related genes has been found in human neointima of restenotic lesions following coronary artery stenting. The functional single nucleotide polymorphism 88G/A is located inside the signal peptide of the interferon gamma receptor 1 and results in an amino acid substitution (Val14Met). The objective of this study was to assess the influence of this polymorphism on restenosis.
Methods: This is a case control study. Subjects of both groups were nondiabetics with stenting in 2 or more vessels and follow-up angiography at 6 months. Cases were selected to have restenosis in at least 2 stented lesions and controls no restenosis at all. Each group consisted of 41 consecutive patients fulfilling these criteria. Genotyping was performed with nested PCR and restriction enzyme analysis.
Results: Cases were 67.6+12.9 years old, controls 67.5+12.0 years. None of the cases or controls were homozygous for the rarer A allele. An excess of A allele carriers was observed among cases with restenosis as compared to controls (Figure).
Conclusions: Interferon gamma receptor 1 88G/A polymorphism may have an influence on the development of restenosis following coronary artery stenting. This may be related to a shift of Th1/Th2 balance described for A allele carriage.



1078-180 Circulatory Endothelial Precursor Cells and Neural-Crest Derived Cells in Human In-Stent Restenosis

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Background: In-stent restenosis (ISR) is a major limitation of interventional cardiology. Circulatory endothelial precursor cells and neural crest derived cells might contribute to neointimal formation as reparation cells. Therefore, the objective of the present study was to evaluate CD34 and CD133 as markers of bone-marrow derived cells, and calcium-binding protein S100, glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE) and nerve growth factor receptor (NGFR) as markers for neural crest derived cells in ISR **Methods:** Atherectomy specimens from 10 patients with coronary (post-stent implantation 6±3) months), 7 patients with peripheral ISR (7±3) and 10 with primary lesions were studied by immunohistochemistry for the presence of each determinant. **Results:** Samples from ISR consistently demonstrated a homogeneous hypercellularity (1212±546 cells/mm^2 in coronary, 1061±257 in peripheral ISR, 665±98 in primary lesions). As a key finding, expression of each marker was significantly increased in ISR compared to primary lesions (each P<0.05; Table). In addition, we found positive correlations for cells of bone-marrow and neural-crest origin. **Conclusion:** The present study demonstrates the presence of intimal cells of bone marrow and neural crest origin in different types of coronary and peripheral atherosclerosis. Their significant expression in human ISR suggests an important role of these cells in this form of accelerated atherosclerosis.

	Coronary ISR	Peripheral ISR	Primary lesion
CD34	5.7±2.5	9.1±6.6	0.6±0.7
CD133	7.2±4.1	6.7±2.0	1.0±0.7
S100	10.6±6.7	8.7±4.1	1.4±1.1
GFAP	8.2±3.9	7.5±1.9	3.1±1.0
NSE	4.1±2.9	4.8±2.1	1.3±1.6
NGFR	4.5±2.3	3.7±2.7	1.1±0.7

1078-181 The Effect of Carvedilol-Loaded BiodyvYsio Stent on Neointimal Hyperplasia in a Porcine Coronary Stent Restenosis Model

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Background and Purpose: Carvedilol is a direct inhibitor of myofibroblast migration in the vascular media and adventitia and exhibits antioxidant properties. We assessed the efficacy of high concentration of carvedilol, loaded with Bio*divYsio*™ DD stent and delivered directly into the vessel wall on the inhibition of neointimal proliferation after stenting in porcine coronary arteries. **Methods:** Total loading amount of carvedilol was determined using 11mm Bio*divYsio*™ Matrix LO stents in different concentration (5mg/mL, 25mg/mL, and 40mg/mL) of carvedilol solution. Twenty-two Bio*divYsio*™ DD stents were implanted at 12 pigs, immediately after being immersed and dried in the different carvedilol or control solution. Quantitative angiographic analysis and histopathologic analysis was done 4 weeks later. **Results:** Total loading amounts of carvedilol on the Bio*divYsio*™ Matrix LO stents were 7±1 μg, 96±18 μg, and 217±34 μg from 5 mg/ml, 25 mg/ml, and 50 mg/ml carvedilol solution, respectively. Twenty-two stents were implanted and overexpanded successfully. No pig was dead during the 4-week observation. On quantitative coronary angiogram, the coronary artery diameters were not significantly different between two groups before stenting or at 4 weeks after stenting.

Table. Histopathologic analysis

	Control	5mg/mL	25mg/mL	40mg/mL	P value
Injury score	1.80±0.63	1.92±0.64	1.92±0.64	1.76±0.59	0.89
EIL area (mm^2)	7.17±0.97	7.15±0.60	6.36±0.43	6.76±1.18	0.067
IIL area (mm^2)	5.35±0.75	6.06±0.58	5.23±0.61	5.57±1.12	0.084
Lumen area (mm^2)	3.72±0.78	5.18±0.68	3.92±0.88	4.44±1.40	0.004*
Neointima (mm^2)	1.63±0.56	0.88±0.30	1.31±0.73	1.13±0.57	0.022*
Diameter stenosis (%)	30.5±10.5	14.6±5.2	25.2±13.4	21.7±14.5	0.016*

* P<0.05 between 5 mg/ml and control solution.
Conclusions: Low dose carvedilol-loaded Bio*divYsio*™ Matrix LO stents inhibit stent restenosis in porcine coronary stent restenosis model.

1078-182 Innate Immunomodulation via Transient Depletion of Monocytes by Liposomal-Alendronate Suppresses Neointimal Formation Following Balloon and Stent Injury in Rabbits

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Background: Inflammation is a major trigger for the reparative events that follow vascular injury. Excessive innate immune response correlates with neointimal hyperplasia and restenosis. We studied the impact of transient systemic inactivation of monocytes and macrophages on intimal hyperplasia following balloon injury and stent deployment.
Methods and Results: Bisphosphonates encapsulated in liposomes are phagocytosed by and specifically inactivate macrophages. Endothelial or smooth muscle cells that do not imbibe these formulations are left intact. Rabbits fed a hypercholesterolemic diet underwent bilateral iliac balloon denudation and stent deployment. Suppression of blood monocytes from 94±18 to 18±15 / ml (p<0.01) was observed 48 hours after injury and intraarterial injection of liposomal alendronate (3 mg/kg). Suppression was transient with return to normal levels a week after injury (flow cytometry for CD14+ cells). The reduction in blood monocytes was associated with reduced infiltration by tissue macrophages (at 6 days, RAM-11 immunostaining), suppressed arterial cellular proliferation (Ki-67 immunostaining) and neointimal formation at 28 days. Intimal area was reduced by 47% (3.88±0.93 to 2.08±0.58 mm2), lumen area was increased by 24% (2.87±0.44 to 3.57±0.65 mm2) and stenosis (%) was reduced by 25% (34± 4 to 25± 4%) (mean±SD, n=16, p<0.01 for all 3 parameters).
Conclusions: Innate immunity triggers vascular repair; and when extreme, as in stented